Water Res. Vol. 19, No. 8, pp. 1047–1058, 1985
Printed in Great Britain.

0043-1354/85 $3.00 + 0.00

AN EVALUATION OF MATHEMATICAL MODELS FOR THE EFFECTS OF pH AND TEMPERATURE ON AMMONIA TOXICITY TO AQUATIC ORGANISMS

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(*Received December* 1984)

Abstract—Available data on the pH and temperature dependence of ammonia toxicity to aquatic organisms were examined and their agreement with various models was evaluated. A model which considers alteration of the relative concentration of un-ionized ammonia at the gill surface failed to adequately describe either pH or temperature dependence. A model that assumes that un-ionized ammonia and ammonium ion are jointly toxic was strongly supported by the data on pH dependence, but could not explain observed temperature dependence. Temperature dependence can be described empirically by a simple log-linear model. The effects of pH and temperature were generally found to be qualitatively and quantitatively similar among fish species.

Key words—ammonia, toxicity, pH, temperature

Various investigators have found the toxicity of ammonia to aquatic organisms to be dependent on pH and temperature. Disagreement and confusion exist regarding how well this dependence adheres to different theoretical models (Lloyd and Herbert, 1960; Tabata, 1962; Robinson-Wilson and Seim, 1975; Armstrong *et al.*, 1978; Szumski *et al.*, 1982). Consideration will be given here to how this dependence can best be mathematically modeled and how similar this dependence is among different species.

Total ammonia in aqueous solution consists of two principal forms, ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). The relative concentrations of these two forms are pH dependent, as described by the following equilibrium expression:

$$K'_a = \frac{[NH_3][H^+]}{[NH_4^+]}. \quad (1)$$

The relative concentrations of the two forms are also temperature dependent, as indicated by the following expression from Emerson *et al.* (1977) for the temperature dependence of K_a:

$$pK_a = 0.09018 + 2729.92/(273.2 + T) \quad (2)$$

where T is temperature in °C. The ratio of un-ionized ammonia to ammonium ion increases by 10-fold for each unit rise in pH and by about 2-fold for each 10°C rise in temperature over the 0–30°C range.

Early investigations of the toxicity of ammonia to aquatic organisms indicated that the toxicity of total ammonia increases with increasing pH (Chipman, 1934; Wuhrman and Woker, 1948; Downing and Merkens, 1955). Because the relative amount of un-ionized ammonia increases with pH, such observations have been interpreted as meaning that un-ionized ammonia is much more toxic than ammonium ion and that un-ionized ammonia constituted the major source of toxicity in these investigations, even though it comprised a small fraction of total ammonia. Based on this interpretation, it has become common practice to express ammonia toxicity on the basis of un-ionized ammonia concentration alone. This mode of expression will be used here. These investigations did not, however, provide sufficient information to adequately quantify the relationship of ammonia toxicity to pH. This was due to test pHs being so highly correlated with un-ionized ammonia concentrations that their effects are inseparable, the use of LT_{50} (the median time-to-death at a constant toxicant concentration) as the descriptor of toxicity rather than LC_{50} (the concentration causing 50% mortality over a fixed duration) and/or an inadequate number or range of pHs which were tested.

Later investigations usually have demonstrated that some pH dependence still exists even when ammonia toxicity is expressed on the basis of un-ionized ammonia concentration, with such LC_{50}s generally increasing with increasing pH (Lloyd and Herbert, 1960; Tabata, 1962; Robinson-Wilson and Seim, 1975; Stevenson, 1977; Armstrong *et al.*, 1978; Thurston *et al.*, 1981; McCormick *et al.*, 1984; Broderius *et al.*, 1985). In contrast, Tomasso *et al.* (1980) found no significant trend of un-ionized ammonia LC_{50}s in the pH 7–9 range. The data from these investigations are plotted in Fig. 1. Except for conversion, where necessary, to units of mg l^{-1} NH_3–N, the LC_{50}s used here are as reported by the in-

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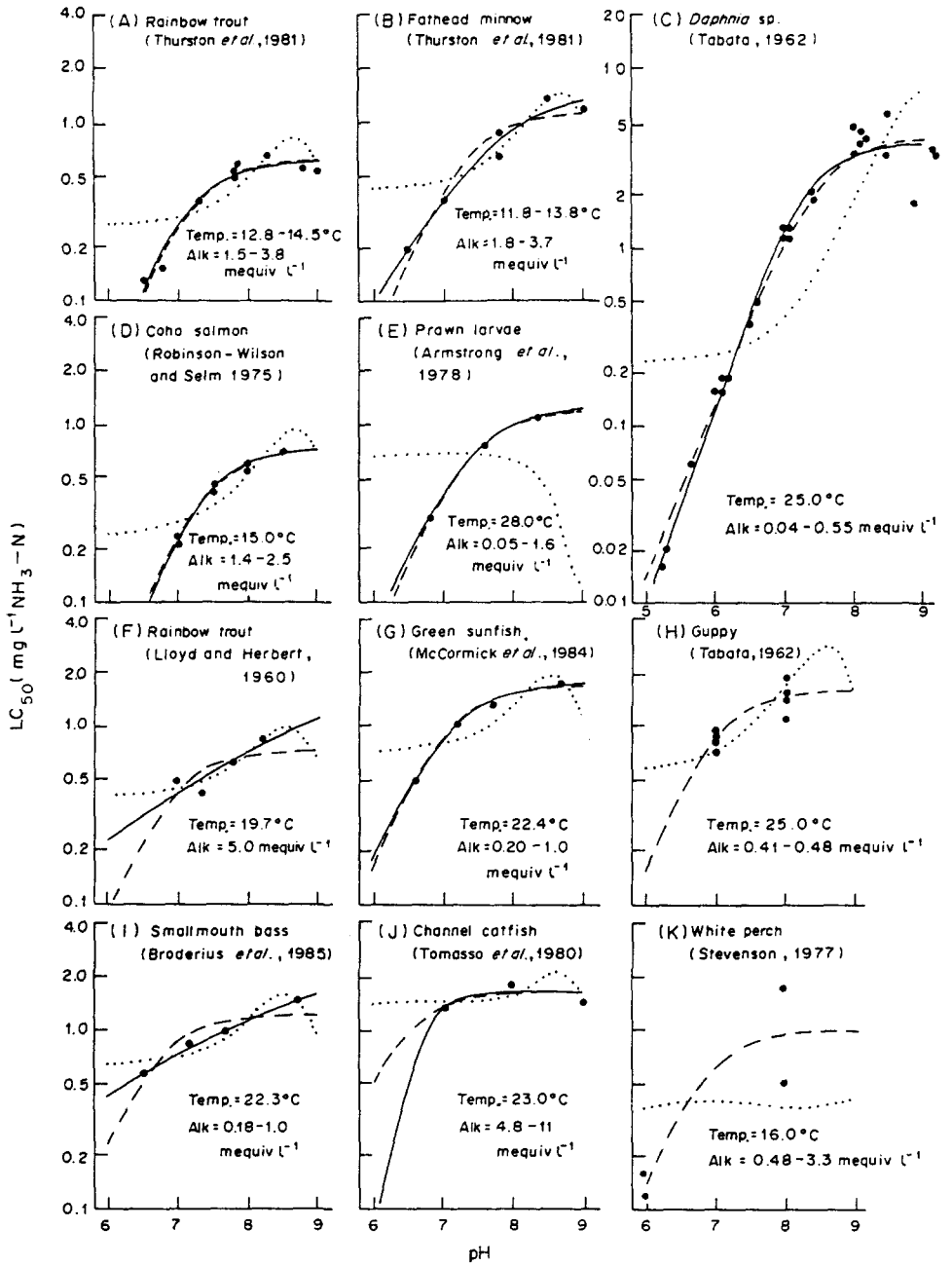

Fig. 1. pH dependence of ammonia toxicity (solid circles denote observed 24 or 96 h LC_{50}s on an un-ionized ammonia basis, solid lines denote empirical pH model, dashed lines denote joint toxicity model and dotted lines denote gill pH model).

vestigators and no attempt was made to evaluate the validity of the LC_{50} estimation procedures or to recalculate LC_{50}s. For the data from McCormick *et al.* (1984), no units conversion was conducted because the units of the LC_{50}s from this source were already mg l^{-1} NH_3–N, rather than mg l^{-1} NH_3 as reported (McCormick, 1984).

Early investigations of the temperature dependence of ammonia toxicity by Powers (1920) and McCay and Vars (1931) showed toxicity to increase with temperature when expressed on the basis of total ammonia, but, because neither study reported pHs, it is not possible to determine how much of this effect may be due to the relative amount of un-ionized ammonia increasing with temperature. Wuhrman and Woker (1953) did show that toxicity, as measured by response times, also increased with temperature when expressed on the basis of un-ionized ammonia, but this information suffers from problems similar to those mentioned above for early studies regarding pH effects. In particular, it is uncertain how much of the effect of temperature is merely due to hastening the metabolic response rather than to altering the incipient toxicity of ammonia.

Later investigations have consistently demonstrated that un-ionized ammonia LC_{50}s increase with increasing temperature, reflecting a decrease rather than an increase, in toxicity (Ministry of Technology, 1968; Hazel *et al.*, 1971; Colt and Tchobanoglous, 1976: Cary, 1976; Roseboom and Richey, 1977; Reinbold and Pescitelli, 1982; Thurston and Russo, 1983; Thurston *et al.*, 1983). The data of these investigators are plotted in Fig. 2. Other than for conversion of units, LC_{50} values here were not changed from those of the authors, except for the case of Hazel *et al.* (1971), who used incorrect stability constants for the calculation of un-ionized ammonia concentrations. For that case, LC_{50}s were adjusted here based on the ratio of un-ionized ammonia concentrations resulting from the correct and incorrect constants. Also, only the freshwater data from this source were used.

Alkalinities are also indicated in Figs 1 and 2 because they are required by one of the models considered below. For the data sets on temperature dependence (Fig. 2), the alkalinities used here were all as reported by the investigators, except for Cary (1976), who did not report alkalinity. For this case, a value of 2 mequiv. l^{-1} was assumed, which is in the

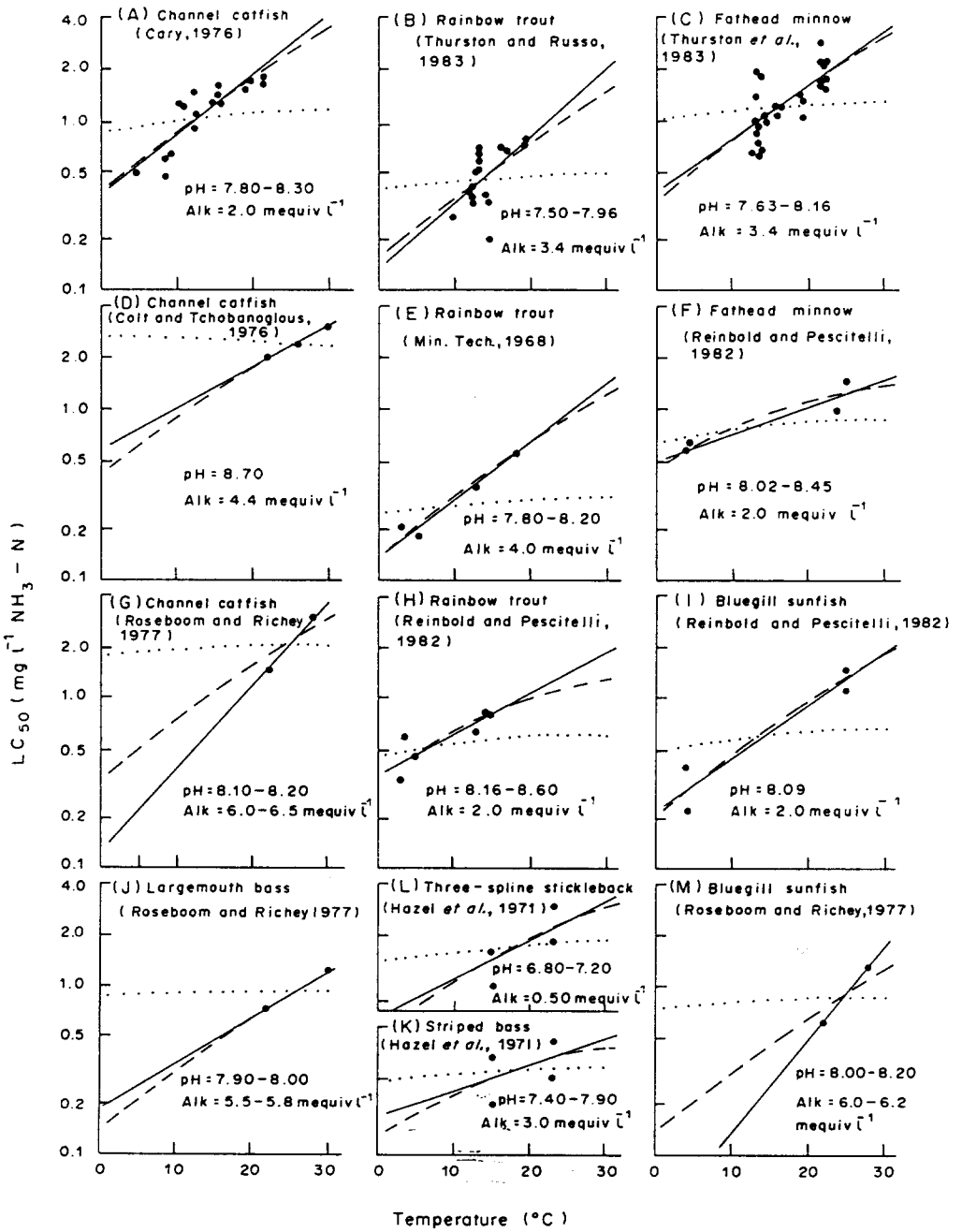

Fig. 2. Temperature dependence of ammonia toxicity (solid circles denote observed 96 h LC_{50}s on an un-ionized ammonia basis, solid lines denote empirical temperature model, dashed lines denote joint toxicity model and dotted lines denote gill pH model).

lower range of alkalinity for the source of water used (San Miguel River, CO) (Willingham, 1984). For the data sets on pH dependence (Fig. 1), pH was modified by addition of CO_2, titration with strong acid or base, or by addition of buffers. Alkalinities used here were as reported by the investigators, except for the data of Robinson-Wilson and Seim (1975), Tabata (1962) and Armstrong *et al.* (1978), all of whom titrated their water to adjust pH but only reported alkalinity for their standard test water. The alkalinities at other than standard pH in these cases were estimated as follows.

(1) Tabata (1962) used sealed bioassay vessels, so under the assumption of no CO_2 loss or gain from the atmosphere, the pH and alakalinity of his standard test water were used to compute the alkalinity adjustment needed to reach any other pH.

(2) Armstrong *et al.* (1978) equilibrated their water with the atmosphere, so by using the partial pressure of carbon dioxide calculated for the pH and alkalinity of their standard test water ($CO_2 = 10^{-3.3}$ atm), the alkalinities of water at equilibrium with this partial pressure at other pHs were estimated.

(3) Robinson-Wilson and Seim (1975) used open vessels without complete equilibration with the air, so neither type of calculation above is possible, but such procedures are comparable to those used by Thurston *et al.* (1981) and Broderius *et al.* (1985) who did report measured alkalinities. The trends of alkalinity with pH reported by these latter investigators were used to approximate that which Robinson-Wilson and Seim would have had.

The ranges of these corrections are indicated in Fig. 1.

FORMULATION OF MATHEMATICAL MODELS

Emprical model for pH dependence

The data sets in Fig. 1(A)–(D) individually contain both a sufficient range of pH and a sufficient number of data points to indicate an appropriate empirical mathematical expression for LC_{50}s vs pH. For all four sets, LC_{50}s appear to approach an asymptotic value at high pH. These data sets also suggest that $\log(LC_{50})$ vs pH is asymptotically linear at low pH. These two properties can be formulated into a single equation as follows:

$$LC_{50} = \frac{LIM}{1 + 10^{SLP(PHT - pH)}} \quad (3)$$

where LIM = asymptotic LC_{50} value at high pH, SLP = asymptotic slope at low pH and PHT = a transition pH.

In Fig. 1(A)–(C) there is some indication that LC_{50}s may actually reach a peak and decline as pH increases to near 9 and beyond, but, given the general uncertainty of the data, the declines cannot be considered of either statistical or practical significance and it is not appropriate to try to incorporate them into an empirical model.

The other seven data sets [Fig. 1(E)–(K)] individually contain insufficient data to indicate the appropriate form for an empirical model. Of these sets, Fig. 1(E) and (G) strongly support the above model. In contrast, Fig. 1(I) seems to lack the asymptotic behavior at pH > 8. Figure 1(F) may indicate that LC_{50}s do not decline as pH is decreased below 7, and Fig. 1(J) shows no appreciable change in LC_{50} over the pH 7–9 range. However, in all of these cases, the amount of information is not enough to discredit the above empirical pH model since the apparent contradictions depend on just one data point and/or on the limited range of pHs used. Finally, Fig. 1(H) and 1(K) contain data points at just two pHs and support the above empirical model merely in that the changes in LC_{50}s between the pHs are similar to those in Fig. 1(A)–(D).

Empirical model of temperature dependence

The data sets in Fig. 2(A)–(C) individually contain both a sufficient range of temperature and a sufficient number of data points to indicate an appropriate empirical mathematical expression for LC_{50}s vs temperature. For all three sets, there is no clear indication that increase of LC_{50}s with temperature is anything but linear in $\log(LC_{50})$ vs temperature. The empirical model adopted for temperature dependence is therefore:

$$LC_{50} = LCR \cdot 10^{SLT(T-20)} \quad (4)$$

where LCR = LC_{50} at a reference temperature of 20°C and SLT = slope of $\log(LC_{50})$ vs temperature.

The other ten data sets [Fig. 2(D)–(M)] individually contain insufficient data to indicate the appropriate form for an empirical model. However, of these sets, the only two [Fig. 2(D) and (E)] that contain data at more than two temperatures strongly support the above empirical model. The remaining data sets [Fig. 2(F)–(M)], each with just two tested temperatures, cannot be used to evaluate this empirical model, but the increases of $\log(LC_{50})$ with temperature in these sets are of similar magnitude over various temperature ranges to that in the larger data sets.

Joint toxicity model

The fact that LC_{50}s on an un-ionized ammonia basis are lower at low pH, where ammonium ion is higher relative to un-ionized ammonia, has led to the suggestion that the pH dependence of ammonia toxicity can be attributed to both un-ionized ammonia and ammonium ion being toxic, but with different potencies (Tabata, 1962; Armstrong *et al.*, 1978). Armstrong *et al.* additionally discussed modes of uptake of the two forms to support the concept of such joint toxicity.

In addition to the fundamental assumption that both un-ionized ammonia and ammonium ion are

toxic, two additional assumptions were made to formulate the model here:

(1) The individual toxicities of the two forms of ammonia are assumed to not depend significantly on pH or temperature. This assumption may well be false, since pH and, especially, temperature are of importance to physiological mechanisms that may alter toxicity. However, a model which intrinsically describes the dependence of a phenomenon on a variable has little utility and is difficult or impossible to verify and calibrate if its parameters depend on the same variable in an unknown fashion, so such an assumption is necessary if this model is to be considered. Model fit will indicate the appropriateness of this assumption.

(2) The toxicities of the two forms of ammonia are assumed to be additive; i.e. toxicity is a function of a weighted sum of the concentrations of the two forms, the weighting factors being indicative of relative potencies. Because un-ionized ammonia and ammonium ion are in rapid equilibrium, additivity should be true if the site of toxic action is internal, where the form of molecule of ammonia is unrelated to its form before being absorbed. More generally, additivity should be true if un-ionized ammonia and ammonium ion have a common site of action and are at equilibrium at the site. However, this assumption should not be considered to be of considerable importance, since deviation from additivity will only affect the degree of curvature in Fig. 1 between the flat portion at high pH and the portion with slope = 1 at low pH. Large deviations from additivity could exist without substantially reducing model fit. Additivity is assumed here in part simply to allow a simple model formulation.

The assumption of additive joint toxicity of any toxicants A and B requires that a mixture of A and B that produces a specified effect obeys the relationship:

$$\frac{[A]}{[A]_e} + \frac{[B]}{[B]_e} = 1 \qquad (5)$$

where $[A]_e$ = the concentration of A required for the effect when B is present in negligible concentration and $[B]_e$ = the concentration of B required for the effect when A is present in negligible concentration.

For the case here, A = un-ionized ammonia, B = ammonium ion, $[B] = [A] \cdot 10^{pK_a - pH}$ and $[A] = LC_{50}$ on an un-ionized ammonia basis. Furthermore, let $[A]_e$, the LC_{50} when only un-ionized ammonia is present in significant concentrations, be denoted as LCU and let the relative toxicity of ammonium ion and un-ionized ammonia ($[NH_3]_e/[NH_4^+]_e$) be denoted as REL. LCU and REL are independent of temperature and pH by assumption (1) above. Rearranging equation (5) and making these substitutions results in the following expression for the joint toxicity model:

$$LC_{50} = \frac{LCU}{1 + REL \cdot 10^{pK_a - pH}}. \qquad (6)$$

This model has obvious relationships to the empirical pH model discussed above. LCU is equivalent to LIM, the asymptotic LC_{50} at high pH. SLP, the slope of $\log(LC_{50})$ vs pH at low pH, is predicted to equal 1 by the joint toxicity model. If SLP = 1, PKT is equivalent to $\log(REL) + pK_a'$. Given these relationships, the support for the empirical model by simple inspection of the data in Fig. 1 also generally applies to the joint toxicity model.

This model also predicts the existence of temperature effects, since pK_a is strongly temperature dependent. Because the relative amount of ammonium ion decreases with increasing temperature, LC_{50}s on an un-ionized ammonia basis are expected to increase with temperature. Furthermore, the slope of $\log(LC_{50})$ vs temperature for the joint toxicity model is approximately constant under the conditions of the tests cited here. This model is therefore qualitatively consistent with the data of Fig. 2 and the empirical temperature model presented above.

Gill pH model

Lloyd and Herbert (1960) suggested that un-ionized ammonia may be the sole significant source of toxicity, with pH dependence being due to expressing LC_{50}s on the basis of bulk water un-ionized ammonia concentrations rather than concentrations at the site of uptake in the gills. Respiration could alter the pH in the gills and thus the relative concentration of un-ionized ammonia. The extent of this alteration may be dependent on the alkalinity and pH of the bulk water, thereby leading to pH dependence of LC_{50}s based on bulk water concentrations even if there would be no such dependence for LC_{50}s based on gill water concentrations. By using calculations similar to those presented below, Lloyd and Herbert demonstrated that toxicity expressed on the basis of calculated concentrations of un-ionized ammonia in the gills did not demonstrate significant trends with pH in their study. Using the same methods, Robinson-Wilson and Seim (1975) also attempted to demonstrate this, but their failure to consider the reduced alkalinity at low pH, as explained above, caused their calculations to be significantly in error. Szumski *et al.* (1982) examined model fit to the data sets of several investigators, but apparently made the same error with regard to alkalinity for some sets, did not properly handle non-carbonate buffers where they occurred, and misreported the pH data from Robinson-Wilson and Seim (1975).

Lloyd and Herbert used the following relationship for the amount of CO_2 released to water as it passes over the gills:

$$CR = DO \cdot RQ \cdot PR \qquad (7)$$

where CR is CO_2 released (in M), DO is the dissolved oxygen concentration of bulk water (in M), RQ is the respiratory quotient and PR is the proportion of influent oxygen removed as water passes over the

gills. They suggested values of 0.8 for RQ and 0.8 for PR for salmonids. They further assumed that this released carbon dioxide is, for practical purposes, immediately in equilibrium with other forms of inorganic carbon (i.e. hydrolysis of carbon dioxide to carbonic acid is fast relative to passage of water over gills) and affected pH accordingly.

There is concern, however, that the hydrolysis of carbon dioxide in water is too slow to permit equilibrium to be achieved within the gills and that carbon dioxide may not be the sole form in which respiratory carbon is released in the gill (Randall, 1970; Broderius *et al.*, 1977; Szumski *et al.*, 1982). Szumski *et al.* assumed that all carbon dioxide was enzymatically hydrolyzed and released as H^+ and HCO_3^-. This allowed the basic mathematical form of the model of Lloyd and Herbert to be retained, merely replacing an assumption of fast external hydrolysis of CO_2 with one of internal hydrolysis. However, this modified assumption is unproven and the question of the form of released respiratory inorganic carbon and its impact on the pH of the gills is uncertain. Also uncertain is the impact on pH of the exchange of other substances, including ammonia itself.

These questions cannot, and need not, be resolved here. Rather, the interpretation of the parameter CR simply needs to be slightly modified. The significance of this parameter, as explained further below, is the impact of hydrolyzed carbon dioxide on the ionic hydrogen mass balance equation. CR should be reinterpreted in terms of this impact, implicitly including only that fraction of respiratory carbon that is hydrolyzed before release or while still in the gills. To further emphasize this interpretation, a new parameter $HR = 2 \cdot CR$ will be used here, representing the hydrogen ion (two for each carbon) associated with hydrolyzed carbon dioxide. If need be, HR can also be considered to incorporate the impact on hydrogen ion mass balance of other exchange processes occurring in the gills. This new parameter should not, however, be considered as changing the model in any fundamental way from that of previous investigators, since it has a simple, rigid equivalence to CR and will have the same impact on pH if estimated by the same methods. CR and HR should be considered to represent the same concept, with the name change simply emphasizing a modification of perspective from carbon release to equivalent hydrogen ion release.

If it is assumed that other chemical reactions are rapid relative to the rate of passage of water across the gills, calculation of the impact of HR (CR) on pH requires the following equilibrium and mass balance expressions:

$$K'_w = [H^+][OH^-] \tag{8}$$

$$K'_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \tag{9}$$

$$K'_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \tag{10}$$

$$K'_B = \frac{[H^+][B^-]}{[HB]} \tag{11}$$

$$ALK = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] + [B^-] \tag{12}$$

$$BTOT = [B^-] + [HB] \tag{13}$$

$$HTOT = [HCO_3^-] + 2[H_2CO_3] + [H^+] - [OH^-] + [HB] \tag{14}$$

where B^- and HB are, respectively, the conjugate acid and base for any non-carbonate buffer of importance (including ammonia itself, if necessary) and HTOT and BTOT are, respectively, the total hydrogen ion and buffer for mass balance purposes.

Alkalinity (ALK) and the concentration of non-carbonate buffer (BTOT) are conservative parameters relative to addition or removal of respiratory carbon. Thus, they can be used as constants in equations describing changes as water passes over the gills and, by combining equations (8)–(13) the concentration of each chemical species can be expressed as a function of only one variable: $[H^+]$. Then, by substituting such expressions into equation (14), HTOT can also be written with $[H^+]$ as the only variable:

$$HTOT = \frac{(ALK + [H^+] - K'_w/[H^+])}{(1 + 2 \cdot K'_2/[H^+])} + \frac{2[H^+]/K'_1 \cdot (ALK + [H^+] - K'_w/[H^+])}{(1 + 2 \cdot K'_2/[H^+])} + [H^+] - \frac{K'_w}{[H^+]} + \frac{BTOT}{(1 + K'_B/[H^+])}. \tag{15}$$

Since the pH_b of the bulk water is measured, equation (15) can be used to calculate $HTOT_b$ for the bulk water. $HTOT_e$ for the water exiting the gills can be computed as $HTOT_b + HR$. The average $HTOT_g$ for water in the gills can be approximated by $(HTOT_b + HTOT_e)/2 = HTOT_b + HR/2$. Equation (15) can then be used again to solve for a representative pH_g of the water in the gills.

Total ammonia is assumed here to remain approximately constant as water passes over the gills. There is no direct evidence to support this assumption, but any decline due to ammonia absorption is unlikely to be substantial since most ammonia in the pH range of concern here is ammonium ion, which has been indicated by toxicity tests to have low toxicity and thus, presumably, low uptake rates. In any event, this assumption is necessary since available data do not permit modeling the decline. As long as the decline is not marked, the impact of this assumption on model fit will be rather minor.

With this assumption, the ratio of the average concentration of un-ionized ammonia in gill water to

that in the bulk water can be approximated as follows:

$$\frac{[NH_3]_g}{[NH_3]_b} = \frac{1 + 10^{pK_a - pH_b}}{1 + 10^{pK_a - pH_g}}. \quad (16)$$

If the average un-ionized ammonia concentration in the gills that results in 50% mortality is some value LCG, the ratio of LCG to LC_{50} should be equal to the above ratio, leading to the following expression for LC_{50}s based on bulk water un-ionized ammonia concentrations:

$$LC_{50} = \frac{LCG}{(1 + 10^{pK_a - pH_b})/(1 + 10^{pK_a - pH_g})}. \quad (17)$$

In applying this model, other investigators have used equation (7) to compute CR and then recomputed LC_{50}s on the basis of average un-ionized ammonia concentrations in the gills to determine how constant they were on this basis. Because of the uncertainty in the estimation and nature of CR (HR), the approach here was to compare observed LC_{50}s with those calculated from equation (17), estimating both LCG and HR as fitted parameters. In so doing, however, HR was constrained to be less than 0.40 mM, representing an extreme value beyond which the model should not reasonably go, based on Lloyd and Herbert's values for PR and RQ and an oxygen concentration of 10 mg l^{-1}.

It should be emphasized that considering HR a fitted parameter is the only fundamental change in the data treatment here from that of Lloyd and Herbert (1960) and Szumski *et al.* (1982). This change will never cause proper measures-of-fit of the model to data to be worse than observed by these other investigators and may well significantly improve fit. Other apparent differences between the treatment presented here and that of others are simply of mathematical equation development which are either arbitrary or needed for proper statistical evaluation of fit.

As for the joint toxicity model, the gill pH model will contain temperature effects due to the effect of temperature on K_a and the other stability constants in the calculations. Szumski *et al.* (1982) have claimed some success using this model to describe temperature dependence of bulk water LC_{50}s. Again, temperature effects other than on equilibrium chemical speciation are assumed here to be nil, since, if this is not true, a fundamental reformulation tantamount to using another model is necessary.

FIT OF MODELS TO DATA

pH dependence of ammonia toxicity

Parameter values for the joint toxicity model and the gill pH model were estimated for the data sets in Fig. 1(A)–(K). Parameter values for the empirical pH model were estimated for the same data sets, except for those in Fig. 1 (H) and (K), for which there were insufficient data. Parameter estimation was by least-squares nonlinear regression, using $\log(LC_{50})$ as the dependent variable and employing the search algorithm of Marquardt (1963). Logarithmic transformation of LC_{50} was conducted because, where indicated for these data sets, the variance of $\log(LC_{50})$, unlike LC_{50}, was approximately homogeneous.

Parameter estimates are included in Table 1. Also reported in Table 1 are the significance level and the R^2 for each regression, computed as described in Draper and Smith (1982). Because the model is nonlinear, these significance levels are only approximate. Where the amount of data precluded computation of a mean square error, it was assumed to be 0.01 with 130 degrees of freedom (=pooled mean square error for best fit model on all other data sets).

The parameter estimates were also used to compute the lines for each model shown in Fig. 1. In computing these lines, the average temperature for each data set was used where needed and alkalinities at

Table 1. Model parameters and goodness-of-fit for data sets on pH dependence of ammonia toxicity

	Empirical model					Joint toxicity model				Gill pH model			
Species (reference)	LIM	PHT	SLP	α	R^2	LCU	REL	α	R^2	LCG	HR	α	R^2
Fathead minnow (Thurston *et al.*, 1983)	1.55	7.79	0.65	<0.01	97	1.17	0.0045	<0.01	93	0.42	0.40	0.06	62
Rainbow trout (Thurston *et al.*, 1983)	0.62	7.16	1.01	<0.01	96	0.63	0.0037	<0.01	96	0.26	0.40	0.03	49
Coho salmon (Robinson-Wilson and Seim, 1975)	0.75	7.35	1.02	<0.01	99	0.75	0.0062	<0.01	99	0.23	0.35	0.01	77
Daphnia sp (Tabata, 1962)	3.87	7.32	1.13	<0.01	98	4.24	0.0178	<0.01	98	0.40	0.40	<0.01	60
Smallmouth bass (Broderius *et al.*, 1985)	2.55	8.31	0.30	0.09	99	1.22	0.0021	0.08	84	0.56	0.10	0.06	88
Green sunfish (McCormic *et al.*, 1984)	1.75	7.04	0.90	0.06	99	1.69	0.0045	<0.01	99	0.62	0.11	>0.20	62
Rainbow trout (Lloyd and Herbert, 1960)	2.40	9.25	0.30	>0.20	74	0.73	0.0029	>0.20	49	0.41	0.40	0.06	88
Macrobrachium rosenbergii (Armstrong *et al.*, 1978)	1.26	7.38	0.93	<0.01	99	1.21	0.0145	0.02	99	0.10	0.40	>0.20	<0
Channel catfish (Tomasso *et al.*, 1980)	1.65	6.68	2.00	>0.20	58	1.66	0.0011	>0.20	50	1.35	0.40	>0.20	27
White perch (Stevenson, 1977)						1.01	0.0019	0.09	83	0.32	0.40	>0.20	6
Guppy (Tabata, 1962)						1.62	0.0052	<0.01	81	0.74	0.05	0.01	80

each pH were obtained by interpolation where needed. Where different types of buffers were used at different pHs, this interpolation also included a blending of buffers appropriate for the desired pH.

Examination of the goodness-of-fit information and fitted lines indicates that the empirical pH model and the joint toxicity model differ very little from each other. This is reflected in the fact that the estimates for SLP for the empirical pH model do not usually differ much from 1.0, at which value the models are equivalent. Furthermore, since the empirical model differs from the joint toxicity model by having an extra parameter, partial F-tests (Draper and Smith, 1982) could be conducted to determine if the additional parameter significantly improves fit. Again, due to nonlinearity, such tests are only approximate. For no data set was there a significant improvement at the $\alpha = 0.10$ level due to using this extra parameter. Thus, because it provides similar fit with fewer parameters, the joint toxicity formulation is preferable to the empirical pH model.

In contrast, the gill pH model is markedly inferior to the joint toxicity model in describing the data. The significance of the regression for the joint toxicity model is highly significant ($< 1\%$) in 6 of the 11 data sets, including all of the four largest sets, and is nonsignificant ($> 10\%$) for only 2 sets. Similarly, the adjusted R^2 is at or above 90% for 6 of the sets and less than 80% for only 2 sets. In contrast, the gill pH model is nonsignificant in 4 sets and is highly significant in only 2 sets. Furthermore, for one of these 2 sets [Fig. 1(C)], the R^2 is much worse than that of the joint toxicity model and it shows apparent deviations from the data. The R^2 for the gill pH model never exceeds 88% and is near or below 60% for 6 of the sets.

In set by set comparisons, the gill pH model shows a markedly better fit (mean square error at least a factor of two smaller) than the joint toxicity model in only 1 set, whereas it shows a markedly poorer fit in 7 sets. Examination of Fig. 1 indicates that the gill pH model predicts certain features of LC_{50} vs pH (major declines at high pH, flattening of trend at low pH) that rarely appear in the data, whereas the typical features of the data are well represented by the joint toxicity model. It should finally be noted that the parameter HR was estimated to be at or near its allowed maximum for 8 of the sets, so if HR was measured or independently estimated, the fits likely would be appreciably worse. Contrasting these high estimates for HR with the greatly lower estimates for the other three sets also indicts model validity.

Temperature dependence of ammonia toxicity

The fits of the models to some of the data sets on pH dependence (Fig. 1) reflect some influence of the temperature differences between data points. This influence is minor because temperature never fluctuated enough to cause effects in the models comparable to the effects of pH. However, for the data sets on temperature dependence of ammonia toxicity (Fig. 2), the pH differences between the data points (as great as 0.5) are not negligible, based on the magnitude of the pH effects observed in Fig. 1. Furthermore, due to either random chance or systematic physical-chemical effects, pH in many of the sets in Fig. 2 is correlated enough with temperature that parameter estimation for the joint toxicity and gill pH models could be perturbed so that some of the effect of temperature on LC_{50}s would be falsely attributed to pH.

To eliminate this problem, the average pH of each data set was used in regression analyses, so that the effects of minor pH changes could not be exaggerated by the parameter estimation techniques. However, this caused another problem in that, if pH and temperature are correlated, some of the apparent temperature effect may be due to pH and will now be falsely attributed to temperature. To minimize this problem, the LC_{50}s in each set in Fig. 2 were adjusted to the average pH of the set as follows:

$$LC_{50adj} = LC_{50} \cdot \frac{1 + 0.004 \cdot 10^{pK_a - pH}}{1 + 0.004 \cdot 10^{pK_a - pH_{avg}}} \qquad (18)$$

where 0.004 is the average estimate for REL from Table 1. Such an adjustment only produces an approximate estimate of what the LC_{50} would have been if determined at the average pH, but because use of the average pH is necessary and because making no adjustment would probably cause greater errors, this procedure was appropriate. In any event, such adjustments of LC_{50}s were usually less than 10% and never greater than 20%, so changes in the analysis are relatively small.

Using adjusted LC_{50}s, parameter values for the empirical temperature model, joint toxicity model, and the gill pH model were estimated for the data sets in Fig. 2. Parameter estimation was as described above for the data sets on pH dependence, except linear regression techniques (Draper and Smith, 1982) were used for the empirical temperature model, because logarithmic transformation causes equation (6) to become linear. For the joint toxicity model, ammonium ion was assumed to be no more toxic than un-ionized ammonia (REL ≤ 1.0). Significance levels and R^2 were also computed as described above. Parameter estimates, significance levels and R^2 for the data on temperature dependence are listed in Table 2. Lines based on these parameter estimates, computed for the average pH and alkalinity, are included in Fig. 2.

Examination of the goodness-of-fit information and fitted lines indicate that the empirical temperature model and joint toxicity model are very similar. As indicated above, this is not surprising because the joint toxicity model over the 0–30°C temperature range at typical pH and alkalinity is not markedly nonlinear. Unlike for the case of pH dependence, the empirical and joint toxicity models do not have different numbers of parameters and using

Table 2. Model parameters and goodness-of-fit for data sets on temperature dependence of ammonia toxicity

Species (reference)	Empirical model				Joint toxicity model				Gill pH model			
	LCR	SLT	α	R^2	LCU	REL	α	R^2	LCG	HR	α	R^2
Fathead minnow (Thurston *et al.*, 1983)	1.60	0.031	<0.01	53	48.8	0.84	<0.01	52	0.71	0.40	0.13	9
Rainbow trout (Thurston and Russo, 1983)	0.81	0.039	<0.01	44	28.6	1.00	<0.01	43	0.28	0.40	>0.20	7
Channel catfish (Cary, 1976)	1.86	0.035	<0.01	76	37.5	1.00	<0.01	77	0.36	0.40	0.07	19
Channel catfish (Colt and Tchobanoglous, 1976)	1.77	0.024	0.06	99	10.6	1.00	0.08	98	2.30	0.01	>0.20	<0
Rainbow trout (Ministry of Technology, 1968)	0.65	0.034	<0.01	98	16.7	1.00	0.01	98	0.16	0.40	>0.20	19
Buegill sunfish (Roseboom and Richey, 1977)	0.48	0.054	0.02	99	13.3	1.00	0.04	78	0.51	0.40	>0.20	3
Channel catfish (Roseboom and Richey, 1977)	1.20	0.049	0.04	99	28.8	1.00	0.06	82	1.20	0.40	>0.20	2
Largemouth bass (Roseboom and Richey, 1977)	0.64	0.027	0.13	99	7.83	0.40	0.13	99	0.90	0.01	>0.20	1
Rainbow trout (Reinbold and Pescitelli, 1982)	1.07	0.024	0.03	74	1.93	0.093	0.03	74	0.13	0.40	>0.20	29
Bluegill sunfish (Reinbold and Pescitelli, 1982)	0.91	0.031	0.04	93	11.9	0.72	0.04	93	0.18	0.40	>0.20	24
Fathead minnow (Reinbold and Pescitelli, 1982)	1.04	0.016	0.05	90	1.78	0.040	0.06	89	0.23	0.40	>0.20	40
Striped bass (Hazel *et al.*, 1971)	0.60	0.015	>0.20	43	1.08	0.014	>0.20	43	0.37	0.40	>0.20	12
Three-spined stickleback (Hazel *et al.*, 1971)	0.47	0.023	0.17	69	1.59	0.011	0.17	69	0.23	0.40	>0.20	19

partial F-tests to select one or the other is not possible.

Even more than was the case for pH effects, it is clear that the gill pH model fails to represent temperature effects. For this model, the regression is nonsignificant for 12 of the 13 data sets and is just barely significant for the other set. In 11 of 13 sets, HR is again at its maximum limit, indicating questionable performance since fit would be worse if HR was directly estimated or restricted to a more reasonable range. In the other 2 sets, HR is at very low values, also indicative of poor model performance. The R^2 is never greater than 40% and usually less than 20%. The lines on Fig. 2 never come close to following data trends. In contrast, for the empirical and joint toxicity models, the regressions are highly significant in all of the three largest data sets and significant in all but three of the remaining sets. The R^2 is always greater than 40% and in all cases the residual error appears to be due to random data scatter rather than any systematic lack of fit.

DISCUSSION

pH dependence of ammonia toxicity

For available data on the pH dependence of ammonia toxicity, the gill pH model was found here to have a generally poor fit and to be markedly inferior to the empirical pH model and joint toxicity model. However, this rejection of the gill pH model does not mean that the concept of alteration of relative un-ionized ammonia concentrations in gills is not valid to some extent; rather, it just means that this alteration cannot explain a major part of the observed variation of LC_{50}s with pH and that other factors are apparently at work that make the gill pH model an inadequate descriptor of this variation.

The joint toxicity model was found here to have a generally good fit to the data and to be preferred to the proposed empirical model because it produces comparable fits with fewer parameters. However, this does not demonstrate that the theoretical concept of joint additive toxicity of un-ionized and ammonium ion is true; rather, the fits just demonstrate that this model is a good empirical descriptor of the data. There are, however, some more positive aspects in the data that suggest that this theoretical concept may be true:

(1) The variation of LC_{50} with pH is very large and has a distinctive shape, thus making a good regression fit difficult to achieve. Despite this, the joint toxicity model, except for the data of Fig. 1(F), produces very good fits and leaves residual error that is little, if any, greater than that expected from the reproducibility of the bioassays. It is difficult to believe that such good fit is only coincidence, especially for a model with just two parameters. Furthermore, the one data set that shows a poor fit to the model does so because of a feature (decline in LC_{50}s as pH is lowered is interrupted) that is based on just one datum and that is in qualitative disagreement with the other data sets. This data set happens also to be the only one in which pH was adjusted by raising CO_2 levels and the different shape may be related to differences in chemical parameters other than ammonia and pH.

(2) If the empirical pH model is applied to all data sets in a pooled regression analysis assuming SLP is the same for all sets, SLP is estimated to equal 1.04, very close to the value (1.00) predicted by the joint toxicity model. Again, it is difficult to believe that such notable agreement to the joint toxicity model is happenstance.

It should be noted that this positive evidence for the joint toxicity model does not also support the assumption of additivity. The plateau at high pH and the slope of 1.0 at low pH are indicative of joint

Table 3. Approximate 95% confidence limits for joint toxicity model parameters for data sets on pH dependence of ammonia toxicity

Species	Reference	LCU (95% CL)	REL (95% CL)
Fathead minnow	Thurston *et al.* (1981)	1.17(0.93–1.52)	0.0045(0.0026–0.0079)
Rainbow trout	Thurston *et al.* (1981)	0.63(0.57–0.70)	0.0037(0.0029–0.0050)
Coho salmon	Robinson-Wilson and Seim (1975)	0.74(0.56–0.99)	0.0062(0.0030–0.0115)
Daphnia sp.	Tabata (1962)	4.24(3.77–4.85)	0.0178(0.0151–0.0214)
Smallmouth bass	Broderius *et al.* (1985)	1.22(0.74–1.66)	0.0020(0.0005–0.0047)
Green sunfish	McCormick *et al.* (1984)	1.69(0.95–2.65)	0.0045(0.0005–0.0106)
Rainbow trout	Lloyd and Herbert (1960)	0.73(0.38–1.12)	0.0029(0.0000–0.0075)
Prawn larvae	Armstrong *et al.* (1978)	1.21(0.96–1.45)	0.0145(0.0088–0.0202)
Channel catfish	Tomasso *et al.* (1980)	1.66(0.56–2.09)	0.0011(0.0000–0.0039)
White perch	Stevenson (1977)	1.01(0.30–2.20)	0.0019(0.0000–0.0043)
Guppy	Tabata (1962)	1.62(1.40–1.80)	0.0052(0.0032–0.0077)

toxicity in general, whether it be by common or different mechanisms and whether it be completely, partially, or non-additive. As plotted in Fig. 1, different joint toxicity models would differ only near the transition pH and could not be easily distinguished given the typical uncertainty of LC_{50} estimates.

Parameter estimates for the joint toxicity model for all 11 data sets on pH dependence are summarized in Table 3. Also included in this table are confidence limits for these estimates. Since a nonlinear regression was involved, these confidence limits are approximate, being based on simulations. Briefly, for each data set, the parameter and error estimates from the regression analysis were treated as true population parameter values and used to randomly generate 1000 sets of LC_{50}s at the same pHs occurring in the data set. These generated data sets were then subject to the same nonlinear regression analysis and the resulting 1000 sets of regression estimates were compared to the "true" values to determine factors that could be applied to the parameter estimates to produce 95% confidence limits.

Temperature dependence of ammonia toxicity

For the available data on the temperature dependence of ammonia toxicity, the gill pH model was found to have a very poor fit and to be markedly inferior to the empirical temperature model and joint toxicity model. Furthermore, unlike for pH dependence, its performance is so poor and its predicted temperature effects are so small, that it cannot be considered to contribute any appreciable amount to the total effect of temperature on ammonia toxicity.

The empirical temperature model and the joint toxicity model both show as good a fit to the data as can be expected, given data uncertainty. Both models fit the data sets about equally well and, since they each contain the same number of fitted parameters, there is no way to statistically select between them using the available data. The empirical model does have the advantages of having a simpler functional form and of not implying the existence of a specific mechanism for which there is no convincing evidence other than overall fit to the data.

There are some reasons, however, for suggesting that joint toxicity of un-ionized and ammonium ion is not principally responsible for the observed effects of temperature:

(1) In Table 2, the estimates for REL, the parameter in the joint toxicity model through which the effect of temperature is exerted, vary widely, ranging from 0.01 to >1. Some variation between species is expected, but such a wide variation in a fundamental parameter discredits the model, especially when it is required to describe a data trend, the slope of $\log(LC_{50})$ vs temperature, which is relatively constant, ranging from 0.015 to 0.054.

(2) The estimates for REL in Table 2 are incompatible with the estimates for REL from the data sets for pH dependence (Table 1). Clearly, joint additive toxicity cannot be solely responsible for the observed dependence on both pH and temperature. It is more reasonable to reject the concept of joint additive toxicity for explaining temperature effects than for pH effects, due to (a) the distinctive features of the pH data and their compatibility with this mechanism, (b) the greater constancy of the estimates for REL from the pH data and (c) the broader possibilities for mechanisms for a temperature effect, given the greater importance, for the experimental ranges here, of external temperature than external pH in regulating internal physiology.

(3) The high (≥ 0.4) values for REL in Table 2 for 9 of the data sets are contrary to the broadly-based assessment in the literature that ammonium ion is much less toxic than un-ionized ammonia.

Of the models considered, the empirical temperature model is the most appropriate to the data. Parameter estimates and confidence limits for this model are summarized in Table 4. Because linear regression analysis was used for this model, these confidence limits were computed as in Draper and Smith (1982) and are exact.

Interspecific differences in model parameter values

The joint toxicity model was formulated here to have one parameter (LCU) which describes the absolute sensitivity of the test organism to ammonia under a reference condition of pH and temperature and another parameter (REL) which regulates the relative change in toxicity of ammonia due to changes in pH and temperature. The confidence limits for LCU in

Table 4. 95% confidence limits for parameters of empirical temperature model for data sets on temperature dependence of ammonia toxicity

Species	Reference	LCR (95% CL)	SLT (95% CL)
Fathead minnow	Thurston *et al.* (1983)	1.60(1.34–1.90)	0.031(0.019–0.043)
Rainbow trout	Thurston and Russo (1983)	0.81(0.53–1.24)	0.039(0.016–0.062)
Channel catfish	Cary (1976)	1.86(1.45–2.39)	0.035(0.024–0.045)
Channel catfish	Colt and Tchobanoglous (1976)	1.77(0.86–3.66)	0.024(0.005–0.053)
Rainbow trout	Ministry of Technology (1968)	0.65(0.39–1.10)	0.034(0.020–0.047)
Bluegill sunfish	Roseboom and Richey (1977)	0.48(0.22–1.04)	0.054(0.008–0.101)
Channel catfish	Roseboom and Richey (1977)	1.20(0.55–2.64)	0.049(0.027–0.096)
Largemouth bass	Roseboom and Richey (1977)	0.64(0.31–1.33)	0.027(−0.008–0.062)
Rainbow trout	Reinbold and Pescitelli (1982)	1.07(0.51–2.24)	0.024(0.004–0.043)
Bluegill sunfish	Reinbold and Pescitelli (1982)	0.91(0.33–2.50)	0.031(0.005–0.057)
Fathead minnow	Reinbold and Pescitelli (1982)	1.04(0.56–1.96)	0.016(0.000–0.033)
Striped bass	Hazel *et al.*, (1971)	0.60(0.30–1.21)	0.015(−0.026–0.066)
Stickleback	Hazel *et al.* (1971)	0.47(0.25–0.88)	0.023(−0.024–0.070)

Table 3 do not overlap for several pairings of species. This suggests that the absolute sensitivity of species to ammonia does vary significantly. Between fish and the two tested crustaceans, there are also significant differences in REL, with confidence limits of pairings of a fish and a crustacean usually not overlapping and with the lowest REL for a crustacean being more than twice the highest REL for a fish. In contrast, pairings among fish show confidence limits for REL to always overlap substantially, suggesting that REL is relatively similar among fish, although the broad confidence limits on this parameter make the degree of similarity uncertain.

The empirical temperature model was also formulated to have one parameter which describes the absolute sensitivity of the test organism under reference conditions (LCR) and another parameter which describes the relative change of sensitivity with temperature (SLT). The confidence limits in Table 4 indicate that the absolute parameter varies significantly among fish species. The relative parameter is again found not to vary significantly among fish species, with the broad confidence limits on the estimates again making the degree of similarity uncertain.

Available data on the pH and temperature dependence of species has therefore shown some quantitative as well as qualitative similarities among species. However, uncertainties in the data, the absence of data at certain pHs and temperatures, and the lack of information on the combined effects of pH and temperature leave some assessments uncertain or impossible to make until appropriate further research is conducted.

Acknowledgements—The author thanks Rosemarie C. Russo and Robert V. Thurston for their encouragement and review of this work. This project was supported by the U.S. Environmental Protection Agency (CR809367 and CR809240).

REFERENCES

Armstrong D. A., Chippendale D., Knight A. W. and Colt J. E. (1978) Interaction of ionized and un-ionized ammonia on short-term survival and growth of larvae, *Macrobrachium rosenbergii*. *Biol. Bull.* **154**, 15–31.

Broderius S. J., Smith L. L. Jr and Lind D. T. (1977) Relative toxicity of free cyanide and dissolved sulfide forms to the fathead minnow *Pimephales promelas*. *J. Fish Res. Bd Can.* **34**, 2323–2332.

Broderius S. J., Drummond R. A., Fiandt J. T. and Russom C. L. (1985) Toxicity of ammonia to smallmouth bass, *Micropterus dolomieui*, as related to pH. *Envir. toxic. Chem.* **4**, 87–96.

Cary G. A. (1976) A report on the assessment of aquatic environmental impact of Union Carbide's Uravan operations, on site toxicity bioassays. Aquatic Environmental Sciences, Union Carbide Corporation, Tarrytown, N.Y.

Chipman W. A. (1934) The role of pH in determining the toxicity of ammonium compounds. Ph.D. thesis, University of Missouri, Columbia, Mo.

Colt J. and Tchobanoglous G. (1976) Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. *Aquaculture* **8**, 209–224.

Downing K. M. and Merkens J. C. (1955) The influence of dissolved oxygen concentrations on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdnerii* Rich.). *Ann. Appl. Biol.* **43**, 243–246.

Draper N. R. and Smith H. (1982) *Applied Regression Analysis*, 2nd edition. Wiley, New York.

Emerson K., Russo R. C., Lund R. E. and Thurston R. V. (1975) Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J. Fish. Res. Bd Can.* **32**, 2379–2383.

Hazel C. R., Thomsen W. and Meith S. J. (1971) Sensitivity of striped bass and stickleback to ammonia in relationship to temperature and salinity. *Calif. Fish. Game* **57**, 138–153.

Lloyd R. and Herbert D. W. M. (1960) The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann. Appl. Biol.* **48**, 399–404.

Marquardt D. W. (1963) An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Ind. appl. Math.* **11**, 431–441.

McCay C. M. and Vars H. M. (1931) Studies upon fish blood and its relation to water pollution. In *A Biological Survery of the St Lawrence Watershed*, pp. 230–233. Supplement to 20th Annual Report, 1930, Biological Survey, New York Conservation Department, Albany, N.Y.

McCormick J. H. (1984) Personal communication. U.S. EPA Environmental Research Laboratory, Duluth, Minn.

McCormick J. H., Broderius S. J. and Fiandt J. T. (1984) Toxicity of ammonia to early life stages of the green sunfish, *Lepomis cyanellus*. *Envir. Poll. Ser. A*. In press.

Ministry of Technololgy (1968) Effects of pollution on fish. In *Water Pollution Research*, pp. 56–65. HMSO, London.

Powers E. B. (1920) Influence of temperature and concentration on the toxicity of salts to fishes. *Ecology* **1**, 95–112.

Randall D. J. (1970) Gas exchange in fish. In *Fish Physiology* (Edited by Hoar W. S. and Randall D. J.), Vol. IV, pp. 253–292. Academic Press, New York.

Reinbold K. A. and Pescitelli S. M. (1982) Effects of cold temperature on toxicity of ammonia to rainbow trout, bluegills, and fathead minnows. Project report, Contract No. 68-01-5832, Illinois Natural History Survey, Champaign, IL.

Robinson-Wilson E. F. and Seim W. K. (1975) The lethal and sublethal effects of a zirconium process effluent on juvenile salmonids. *Water Res. Bull.* **11**, 975–986.

Roseboom D. P. and Richey D. L. (1977) Acute toxicity of residual chlorine and ammonia to some native Illinois fishes. Report of Investigation 85, Illinois State Water Survey, Urbana, IL.

Stevenson T. J. (1977) The effects of ammonia, pH, and salinity on the white perch, *Morone americana*. Ph.D. thesis, University of Rhode Island, Kingston, RI.

Szumski, D. S., Barton D. A., Putnam H. D. and Polta R. C. (1982) Evaluation of EPA un-ionized ammonia toxicity criteria. *J. Wat. Pollut. Control Fed.* **54**, 281–291.

Tabata K. (1962) Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbonic acid. *Bull. Tokai Reg. Fish Res. Lab.* **34**, 67–74 (translated).

Thurston R. V. and Russo R. C. (1983) Acute toxicity of ammonia to rainbow trout (*Salmo gairneri*). *Trans. Am. Fish. Soc.* **112**, 696–704.

Thurston R. V., Russo R. C. and Phillips G. R. (1983) Acute toxicity of ammonia to fathead minnows (*Pimephales promelas*). *Trans. Am. Fish. Soc.* **112**, 705–711.

Thurston R. V., Russo R. C. and Vinogradov G. A. (1981) Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. *Envir. Sci. Technol.* **15**, 837–840.

Tomasso J. R., Goudie C. A., Simco B. A. and Davis K. B. (1980) Effects of environmental pH and calcium on ammonia toxicity in channel catfish. *Trans. Am. Fish. Soc.* **109**, 229–234.

Willingham T. (1984) Personal communication. U.S. EPA Region VIII Office, Denver, CO.

Wuhrman K. and Woker H. (1948) Contributions to the toxicology of fishes. II. Experimental investigations on ammonia- and hydrocyanic acid poisoning. *Schweiz. Z. Hydrol.* **11**, 210–244 (translated).

Wuhrman K. and Woker H. (1953) On the toxic effects of ammonia and cyanide solutions on fish at different oxygen tensions and temperatures. *Schweiz. Z. Hydrol.* **15**, 235–260 (translated).